

METHOD FOR SOFTENING LINES AND RELAXING THE SKIN WITH
ADENOSINE AND ADENOSINE ANALOGUES

Reference to Prior Applications

5 This application claims priority to U.S.
provisional application 60/432,634 filed December 12,
2002, and to French patent application 0214828 filed
November 26, 2002, both incorporated herein by
reference.

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Field of the Invention

 The present invention relates to a method for
softening lines and/or relaxing the skin, and/or
relaxing facial features, comprising topical
15 application to the skin of a composition comprising at
least one compound selected from the group consisting
of adenosine and analogues of adenosine, in a
physiologically acceptable medium. Particular uses of
the invention composition include the decreasing of
20 wrinkles, the reduction in laugh lines, the reduction
in frown lines, etc.

 It also relates to the use of at least one
compound as defined above, in a composition suitable
for topical application to the skin, as an agent
25 intended to soften lines and/or relax the skin and/or
relax facial features.

 Additional advantages and other features of the
present invention will be set forth in part in the

description that follows and in part will become
apparent to those having ordinary skill in the art upon
examination of the following or may be learned from the
practice of the present invention. The advantages of
5 the present invention may be realized and obtained as
particularly pointed out in the appended claims. As
will be realized, the present invention is capable of
other and different embodiments, and its several
details are capable of modifications in various obvious
10 respects, all without departing from the present
invention. The description is to be regarded as
illustrative in nature, and not as restrictive.

Background of the Invention

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Women and, increasingly, men have a tendency to
want to appear young for as long as possible, and so
they seek to tone down signs of ageing in the skin,
primarily wrinkles and fine lines. Thus,
20 advertisements and the fashion industry promote
products intended to keep the skin radiant and wrinkle-
free, the trade marks of a young skin, for as long as
possible. Furthermore, physical appearance has an
effect on psyche and/or morale.

25

Until now, wrinkles and fine lines have been
treated using cosmetic products containing active
ingredients that have an effect on the skin, for
example by moisturizing it or improving cell renewal,

or by encouraging the synthesis of collagen from which cutaneous tissue is formed, or by preventing its degradation.

Although such treatments can have an effect on wrinkles and fine lines due to chronological or intrinsic ageing, and on those cells due to photo-ageing, they do not have any effect on expression lines.

Expression lines are produced by mechanisms that differ from those generating lines due to ageing.

More precisely, they are produced by the stress exerted on the skin by the facial muscles which produce facial expressions. Depending on the shape of the face, the frequency of expressions and the existence of any tics, they can appear in childhood. Age and some environmental factors such as exposure to the sun do not have any effect on their genesis but can make them deeper and render them permanent.

Expression lines are characterized by the presence of furrows at the periphery of the orifices, namely the nose (nasogenic furrows), the mouth (parabuccal lines and bitterness lines) and the eyes (crows feet) around which the facial muscles are located, and also between the eyebrows (glabellar lines or frown lines) and on the forehead.

Until now, the only routine means for dealing with expression lines are botulinum toxin, which is injected into the glabellar lines (see J. D. Carruthers

et al, J. Dermatol. Surg. Oncol., 1992, 18, pp 17-21)
and degradable collagen-based, hyalruonic acid-based or
polylactic acid-based implants.

Further, as an alternative to those medical
5 techniques requiring the services of a skilled
practitioner, the Applicant has proposed a number of
compounds that can provide a myorelaxing effect when
topically applied to the skin and which allow
expression lines to be dealt with in a different
10 manner. Examples of such compounds that can be cited
are antagonists for receptors associated with calcium
channels (French application FR-A-2 793 681), and in
particular manganese and its salts (FR-A-2 809 005) and
alverine (FR-A-798 590); and agonists for receptors
15 associated with the chlorine channel, including glycine
(EP-A-0 704 210) and certain extracts of *Iris pallida*
(FR-A-2 746 641).

However, there is still a need for effective
compounds for relaxing the skin with a view to
20 smoothing or toning down expression lines.

Brief Description of the Figure

Figure 1 illustrates the contraction over time
25 of an equivalent dermis treated with adenosine.

Detailed Description of the Invention

As noted above, the present invention relates to a method for softening lines and/or relaxing the skin, and/or relaxing facial features, comprising topical application to the skin of a composition comprising at least one compound selected from the group consisting of adenosine and analogues of adenosine, in a physiologically acceptable medium.

Particular uses of the invention composition include the decreasing of wrinkles, the reduction in laugh lines, the reduction in frown lines, etc.

The inventor has surprisingly discovered that adenosine and its analogues can satisfy the above need for effective compounds for relaxing the skin with a view to smoothing or toning down expression lines, relaxing the skin, relaxing facial features, decreasing wrinkles, reducing laugh lines, reducing frown lines, etc. More precisely, the inventor has demonstrated that adenosine and its analogues can relax the dermal contractile cells which are believed to be involved in the genesis of expression lines, etc. It is believed that the phenotype of certain fibroblasts located along the tension lines created under the effect of contraction of facial muscles when making a facial expression is progressively modified under the effect of said contractions, endowing said fibroblasts with particular contractile properties. Relaxing those

cells would thus combat expression lines. Of course,
the inventor is not bound by any theory of operation.

In the pharmaceutical field, adenosine is
administered orally or intravenously as a vasodilator
5 and an anti-arrythmic.

In the cosmetics field, it has been suggested,
in United States documents US-A-6 423 327 and
US-2003/044439, that adenosine or an analogue of
adenosine be used in a composition that is topically
10 applied to the skin to improve skin condition and in
particular to combat lines, skin laxity, skin dryness
and pigmentary blemishes. It was indicated that
adenosine increases the size of fibroblasts and
increases the synthesis of proteins by fibroblasts.

15 In the same field, documents WO-A-01/43704,
US-A-3 978 213, US-A-5 371 089, German patents
DE-195 45 107 and DE-200 22 691 disclose compositions
with an anti-ageing effect comprising adenosine or an
adenosine analogue.

20 None of those documents suggests that adenosine
could have a relaxing effect on contractile
fibroblasts.

Thus, the present invention provides a method
for softening lines and/or relaxing the skin,
25 comprising topical application to the skin of a
composition comprising at least one compound selected
from adenosine and an analogue of adenosine, in a
physiologically acceptable medium.

It also concerns the use of at least one compound as defined above in a composition adapted for topical application to the skin as an agent for softening lines and/or relaxing the skin.

5 The present invention further provides a method for softening lines and/or relaxing the skin, comprising topical application to the skin of an amount of a composition comprising at least one compound selected from the group consisting of adenosine and
10 analogues of adenosine, in a physiologically acceptable medium, effective to provide a relaxing effect on contractile fibroblasts.

Adenosine analogues that can be used in accordance with the invention and can be cited as
15 particularly useful herein include agonists of adenosine receptors and compounds increasing intra- or extra-cellular adenosine levels.

Examples of adenosine analogues include: 2'-deoxyadenosine; 2',3'-isopropoylidene adenosine;
20 toyocamycin; 1-methyladenosine, N-6-methyladenosine; adenosine N-oxide; 6-methylmercaptapurine riboside; 6-chloropurine riboside; 5'-adenosine monophosphate; 5'-adenosine diphosphate and 5'-adenosine triphosphate.

Other adenosine analogues include agonists of
25 adenosine receptors, including phenylisopropyl adenosine (PIA), 1-methylisoguanosine, N⁶-cyclohexyl adenosine (CHA), N⁶-cyclopentyl adenosine (CPA), 2-chloro-N⁶-cyclopentyladenosine, 2-chloroadenosine, N⁶-

phenyladenosine, 2-phenylaminoadenosine, MECA, N⁶-phenethyladenosine, 2-p-(2-carboxyethyl)-phenethyl-amino-5'-N-ethylcarboxamido-adenosine (CGS-21680), N-ethylcarboxamido-adenosine (NECA), 5'-(N-cyclopropyl)-carboxamidoadenosine, DPMA (PD 129,944) and metrifudil.

Other adenosine analogues include compounds which increase the intracellular concentration of adenosine such as erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) and iodotubercidin.

Other adenosine analogues include salts and esters of adenosin.

Adenosine is preferred for use in the present invention. It is commercially available in the form of a powder from PHARMA WALDHOF.

The composition in accordance with the invention is preferably intended to be applied to zones of the face or forehead marked with expression lines and/or to persons having expression lines.

The lines concerned are preferably selected from: crow's feet, nasogenic furrows, inter-eyebrow lines and forehead lines.

The quantity of adenosine and/or adenosine analogue for use in accordance with the invention is a function of the desired effect and can thus vary widely. To provide an order of magnitude, the composition of the invention can comprise 0.001% to 10% by weight, preferably 0.01% to 1% by weight of

adenosine and/or adenosine analogue with respect to the total composition weight.

The composition of the invention is suitable for topical application to the skin and thus it
5 contains a physiologically acceptable medium, i.e. a medium that is compatible with the skin. Such media can comprise water, C1-C8, preferably C1-C4, alcohols, etc.

This composition can be fluid to a greater or lesser extent and can have the appearance of a white or
10 coloured cream, a pommade, milk, serum, paste or foam. It can also be in the form of a solid, in particular in the form of a stick. It can be used as a skin care product and/or as a skin makeup product.

The composition of the invention can be in any
15 form, including any of the galenical forms that are normally used in the cosmetics field; in particular, it can be in the form of an aqueous, possibly gelled solution, a lotion type dispersion which may be a two-phased dispersion, an emulsion obtained by dispersing
20 an oily phase in an aqueous phase (O/W) or vice versa (W/O), a triple emulsion (W/O/W or O/W/O) or an ionic and/or nonionic vesicular type dispersion. Said compositions are prepared using the usual methods. Preferably, a composition in the form of an oil-in-
25 water emulsion is used in the present invention.

When the composition used in the invention is an emulsion, the proportion of oily phase can be from 5% to 80% by weight, preferably 5% to 50% by weight

with respect to the total composition weight. Oils, emulsifying agents and co-emulsifying agents used in the composition in the emulsion form are selected from those conventionally used in the field under

5 consideration. The emulsifying agent and co-emulsifying agent are present in the composition in a proportion of 0.3% to 30% by weight, preferably 0.5% to 20% by weight with respect to the total composition weight.

10 Oils that can be used in the invention that can be cited are hydrocarbons of mineral or synthetic origin (Vaseline oil, isohexadecane), oils of plant origin (apricot kernel oil, the liquid fraction of karite butter oil, avocado, soya oil), oils of animal
15 origin (lanolin), synthesized oils (perhydrosqualene, pentaerythrityl tetraoctanoate), silicone oils (cyclopentasiloxane and cyclohexasiloxane) and fluorinated oils (perfluoropolyethers). It is also possible to use fatty alcohols (cetyl alcohol or
20 stearyl alcohol), fatty acids (stearic acid) or waxes (carnauba wax, ozokerite, beeswax) as the oily materials.

Examples of emulsifying and co-emulsifying agents that can be used in the invention that can be
25 cited are esters of fatty acids and polyethylene glycol such as PEG-100 stearate and PEG-20 stearate and esters of fatty acids and glycerin such as glyceryl stearate.

The composition of the invention can also contain adjuvants, including those that are normal in the cosmetics field, such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active ingredients, preservatives, antioxidants, solvents, perfumes, fillers, filters, pigments, odour absorbers and colorants. The quantities of these different adjuvants are those that are conventionally used in the field under consideration, for example 0.01% to 20% of the total composition weight. Depending on their nature, such adjuvants can be introduced into the oily phase, into the aqueous phase or into the lipid vesicles. In all cases, said adjuvants and the proportions thereof should be selected so that they do not deleteriously affect the desired properties of the adenosine/analogue.

Particular examples of hydrophilic gelling agents that can be cited are carboxyvinyl polymers (carbomers), acrylic copolymers such as acrylate/alkylacrylate copolymers, polyacrylamides, polysaccharides, natural gums and clays, and examples of lipophilic gelling agents that can be cited are modified clays such as bentonites, metal salts of fatty acids, hydrophobic silicon and polyethylenes.

Examples of preservatives that can be cited are esters of para-hydroxybenzoic acid, octane-1,2-diol, 3-iodo-2-propynyl-butylcarbamate, phenoxyethanol and chlorhexidine gluconate.

Examples of fillers that can be cited are polyamide (Nylon) particles; polymethyl methacrylate microspheres; ethylene-acrylate copolymer powders; expanded powders such as hollow microspheres and in particular, microspheres formed from a terpolymer of vinylidene chloride, acrylonitrile and methacrylate and sold by Kemanord Plast under the trade name EXPANCEL; powders of natural organic materials such as starch powders, in particular corn starch, wheat starch or rice starch, which may or may not be cross-linked, such as starch powder cross-linked with octenyl succinate anhydride; silicone resin microbeads such as those sold by Toshiba Silicone under the trade name TOSPEARL; silica; metal oxides such as titanium dioxide or zinc oxide; mica; and mixtures thereof.

As indicated above, the composition of the invention can also include UVA and/or UVB filters in the form of organic or inorganic compounds, the latter optionally being coated to render them hydrophobic.

More particularly preferred organic filters are selected from the following compounds (cited using the CTFA nomenclature): Ethylhexyl Salicylate, Ethylhexyl Methoxycinnamate, Octocrylene, Phenylbenzimidazole Sulfonic Acid, Benzophenone-3, Benzophenone-4, Benzophenone-5, 4-Methylbenzylidene camphor, Terephthalylidene Dicamphor Sulfonic Acid, Disodium Phenyl Dibenzimidazole Tetra-sulfonate, 2,4,6-tris-(diisobutyl-4'-aminobenzalmalonate)-s-triazine,

during facial expressions. Under these conditions, cells spontaneously express tensile forces which induce retraction of the collagen gel. This results in a reduction in the total surface area of the equivalent dermis over time. Measuring that surface area allows the relaxation effects of substances that have been brought into contact with the equivalent dermis to be determined.

b) Protocol

Two series of 3 attached equivalent dermises containing normal human fibroblasts were prepared: a control series with no treatment, and a series treated with adenosine (0.01%). The experiment was carried out three times.

The skin equivalents were prepared as described by Asselineau et al, Exp. Cell. Res., 1985, 159, 536-539; Models in Dermatology, 1987, vol 3, pp 1-7, in the following proportions:

MEM medium (1.76X) with or without adenosine:	45%
Foetal calf serum:	9%
NaOH (0.1 N):	5%
Acetic acid (1/1000):	4%
Collagen:	26%
Fibroblasts:	11%

The treated equivalent dermis differed from the control equivalent dermis in that 0.01% of adenosine had been added.

The collagen used was type I collagen (commercial solution), but it was also possible to use type III or IV collagen. It was extracted from rat tails or calf skin by acid hydrolysis and stored in an acidic medium at +4°C; it polymerizes naturally by heating to 37°C and by reducing the acidity. The collagen had been dialyzed against successive baths of water + acetic acid.

The following protocol was employed: the following were introduced into a sterile tube: 1.76X MEM medium in the presence of additives (glutamine 1%, non essential amino acids 1%, sodium pyruvate 1%, fungizone 1% and penicillin/streptomycin 1%), foetal calf serum, 0.1 N sodium hydroxide NaOH. Fibroblasts isolated from human skin explants were then added in a concentration of 1.4×10^5 cells per ml of culture medium.

A 1/1000 vol/vol mixture of collagen in acetic acid was then slowly added by pouring it down the tube wall so that the appearance of a whitish cloud was observed.

The ensemble was then carefully mixed and distributed into the wells of a 12-well culture plate (Costar, reference 3512) in an amount of 0.5 ml of mixture per cm^2 . The culture plate was placed in an incubator at 37°C with 5% CO_2 .

Once formed after polymerizing the collagen, the equivalent dermises were left adhering to the

culture support for 3 days then detached from the support so that contraction could commence. Said attached equivalent dermises were removed from the incubator to record images with a view to measuring
5 their surface area at each point of the contraction kinetics (0, 4, 8 and 24 hours). They were immediately replaced in the incubator between each measuring point.

The spontaneous contraction of the treated (with adenosine) equivalent dermises and control (no
10 adenosine) equivalent dermises was carried out by measuring their surface area at different times after the onset of spontaneous contraction.

To this end, a digital image was acquired for each treated or untreated equivalent dermis using a
15 camera (CCD Camera - Iris Sony DXC - 107P) and the surface area was then calculated for each image using an image analysis system (Zeiss Axiovision 3.0). This surface area measurement corresponded to a percentage contraction which equals the ratio of the surface areas
20 in accordance with the formula:

$$\% \text{ contraction} = (S_p - S_i) / S_p \times 100$$

in which:

"Sp" represents the surface area of one well in the culture plate; it corresponds to the total surface
25 area of the equivalent dermis before contraction;

"Si" represents the surface area of the equivalent dermis at the instant i in the contraction kinetics.

c) Results

As shown in the accompanying Figure, the degree of contraction of the control equivalent dermis was 32% four hours after having been detached from its support.

5 It increased to 42% after eight hours and reached 54% after twenty-four hours.

Adenosine reduced this contraction percentage by 6.4% after four hours, 10.5% after eight hours and 12.7% after twenty-four hours compared with the
10 control.

Thus, this test demonstrates that adenosine causes less contraction in an equivalent dermis, and thus has a relaxing effect which can be exploited in the preparation of compositions with a dermo-relaxant
15 effect. As used herein, the relaxing effect is noted any time less contraction is observed, including less than 1%, 1%, 3%, 5%, etc.

EXAMPLE 2: Cosmetic composition

This composition was prepared in a manner that
20 was conventional for the skilled person. The quantities given in this example are indicated as percentages by weight.

Adenosine	0.10%
Stearic acid	3.00%
Mixture of glyceryl mono- stearate and polyethylene glycol stearate (100 OE)	2.50%
Polyethylene glycol stearate (20 OE)	1.00%
Cyclopentadimethylsiloxane	10.00%
Fillers	3.00%
Vegetable oils	7.00%
Synthetic oils	6.00%
Preservatives	1.20%
Dimethylsiloxane, oxyethylenated (16 OE) with methoxy extremities	1.00%
Silicone gum	0.20%
Acrylic copolymer, in reverse emulsion (Simulgel 600 from SEPPIC)	1.70%
Stearyl alcohol	1.00%
Water	qsp 100%

This cream was intended for application to the face and forehead to soften lines and relax the skin of the face.

5 The above written description of the invention provides a manner and process of making and using it such that any person skilled in this art is enabled to make and use the same, this enablement being provided in particular for the subject matter of the appended

10 claims, which make up a part of the original description and including a cosmetic method for softening lines and/or relaxing the skin, and/or for relaxing facial features ("detendre les traits") comprising topical application to the skin of a

15 composition comprising at least one compound selected from adenosine and an analogue of adenosine, in a

physiologically acceptable medium. Similarly, the invention composition can decrease wrinkles, reduce laugh lines, reduce frown lines, etc.

Preferred embodiments of the invention similarly fully described and enabled include use of at least one compound selected from adenosine and an adenosine analogue in a composition suitable for topical application to the skin, as an agent intended to soften lines and/or relax the skin, and the use of the invention compositions in an amount effective to provide a relaxing effect on contractile fibroblasts.

As used above, the phrases "selected from the group consisting of" and "selected from" include mixtures of the specified materials.

All references, patents, applications, tests, standards, documents, publications, brochures, texts, articles, etc. mentioned herein are incorporated herein by reference. Where a numerical limit or range is stated, all values and subranges therewithin are specifically included as if explicitly written out.

The above description is presented to enable a person skilled in the art to make and use the invention, and is provided in the context of a particular application and its requirements. Various modifications to the preferred embodiments will be readily apparent to those skilled in the art, and the generic principles defined herein may be applied to other embodiments and applications without departing

from the spirit and scope of the invention. Thus, this invention is not intended to be limited to the embodiments shown, but is to be accorded the widest scope consistent with the principles and features disclosed herein.